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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF:

DYMECKI

Appln. No. 08/866,279 Group Art Unit: 1632

Filed: May 30, 1997 Examiner: A.-M. Baker

FOR: USE OF FLP RECOMBINASE IN MICE

* * *

June 6, 2000

RESPONSE UNDER 37 CFR § 1.116

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Box AF

Sir:

Responsive to the Advisory Action mailed April 21, 2000 (Paper No. 17), consideration of the following remarks is respectfully requested.

Claims 1-49 are pending. Reconsideration and allowance are respectfully requested.

The Examiner's indication that the claim amendments of March 14, 2000 will be entered and overcome the rejection under Section 112, second paragraph, is acknowledged.

Claims 1-49 were rejected under Sections 102 and 103. Applicant traverses.

The Advisory Action states:

> "The declaration is unconvincing because it leaves unclear whether the method employed in the specification and declaration would be expected to produce a functional recombination wherein the recombined gene is intact and functional. The experiments described in the instant specification are parallel to those described in the declaration of Dr. Hammer, but no recombination was detected in the experiments carried

> out by Dr. Hammer. Thus, the information provided in the declaration raises the question of whether the transgenic mice described in the instant specification can be expected to produce a functional gene upon recombination. Functional recombination is essential to the operability of the invention."

Firstly, it is recombination between Flp-recognition sequences catalyzed by functional recombinase that is essential to operability of the claimed invention. See claim 1. Flp-mediated recombination resulting in a functional gene is only one objective of the invention. It is not essential to the invention's operability because cell marking and lineage tracing can be accomplished by recombination of an integrated DNA substrate and, for example, either direct detection of the of the recombined DNA substrate or deletion of a histochemical marker. See description of generation of *Wnt1::FLP* transgenic mice on p. 45 of the specification and results reported in Dymecki (Develop. Biol., 201:57-65, 1998 and made of record on Form PTO-1449 dated February 3, 2000).

In Example 2, Applicant showed that (a) recombination was only detected in mice containing recombinase and the Flp-recognition sequences and (b) recombination was site specific. See pages 39-40 of the specification. Recombination could be detected despite the poor expression of h*ACTB* regulatory sequences driving *lacZ* and the inability to stain cells with XGal. See p. 45 of the specification.

In claim 4, the result of recombination is a transgenic mouse containing at least two diploid cells with different numbers of Flp-recombination sequences (i.e., chimeric or

mosaic mice) is recited. Thus, an organ or other tissue showing chimerism or mosaicism in the number of Flp-recombination sequences may be used to trace cell lineages. See pages 4-5 of the specification ("Cell lineages may be traced independent of gene activity, by monitoring differences in the integration site of the Flp substrate").

A functional recombination product (i.e., activation of gene expression by recombination) is also not necessary to achieve the objectives of translocation between chromosomes, excision of a gene to create a null mutation, and transgene insertion into the genome. See pp. 5-6 of the specification.

Thus, the specification shows that recombination mediated by Flp recombinase is site-specific and that production of a functional recombined gene product is not essential for operability of the invention.

Secondly, the Declaration of Dr. Robert E. Hammer clearly shows that the prior art's inability to produce the claimed invention was not due to the inability of a functional, recombined gene to cause transgenic mice to grow larger.

¶12 of the Declaration explains that a complete human growth hormone (hGH) gene driven by the mouse metallothionein-I (MT) in a transgenic mouse will cause the mouse to grow significantly larger. Attachment F of the Declaration shows that homologous recombination and formation of a complete hGH gene thereby can be conveniently detected in this assay.

¶13 of the Declaration states that the presence of a Flp-recognition site in the third intron of the hGH gene has no

effect on MT-hGH expression. A transgenic mouse containing construct #131 (i.e., the expected product of site-specific recombination mediated by Flp is a complete hGH gene) grew significantly larger.

¶15 of the Declaration describes a control in which recombination between coinjected DNA molecules were substrates for homologous recombination. Such recombination also shows that a functional gene (i.e., a complete hGH gene) can be detected by increased size of the transgenic mouse.

Dr. Hammer did not depend solely on obtaining transgenic mice of increased size to detect Flp-mediated recombination. The production of double-transgenic mice with a repeated array of trangenes (construct #165), each containing a Flp-recognition site, and a Flp recombinase transgene (construct #330) is shown in ¶19. Flp-mediated recombination in such double-transgenic mice is not detectable by increased size of the mice because the portion of the hGH gene missing from construct #165 is not provided in this experiment. Instead, a change in the length of the repeat array would result from recombination but ¶20 of the Declaration concludes that no recombination between Flp-recognition sites was detected.

In contrast, ¶21 of the Declaration describes failed attempts to detect recombination by production of transgenic mice of increased size.

As emphasized in ¶22 of the Declaration, it is the Flp recombinase transgene that must be functional. The expected result of Flp-mediated recombination in ¶21 relied on the

detection of a transgenic mouse of increased size, but the expected result in ¶20 did not rely on the production of a functional gene.

Thus, the question raised in the Advisory Action (i.e., whether the transgenic mice described in this specification can produce a functional gene upon recombination) does not bear on the persuasiveness of the Declaration of Dr. Robert E. Hammer. As explained above, the controls described in the Declaration establish that a complete hGH gene (i.e., the functional recombination product expected by the claimed invention) would cause a transgenic mouse to grow larger.

If this issue is maintained by the Examiner, she is requested to explain with particularity (a) why production of a functional gene must result from operation of the claimed invention when gene activation or inactivation are both results envisioned in the specification and (b) why a person skilled in the art would doubt that a functional gene can be produced by the claimed invention.

Finally, Applicant respectfully repeats the request for review of the formal drawings submitted in this application and return by the Official draftsperson of Form PTO-948 in the next Action.

For the above reasons, Applicant respectfully requests withdrawal of the rejections under Sections 102 and 103.

Having fully responded to all claim rejections, it is submitted that the pending claims are allowable and an early Notice to that effect is earnestly solicited. If further

information is needed, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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